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1 **Performance characteristics of the VIDAS® ANTI-HEV IgM and IgG assays**

2

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26 **SUMMARY**

27 **Background.** Several unautomated anti-HEV diagnostic tests are presently available.

28 **Objective.** We have evaluated the performance of the new automated VIDAS® ANTI-HEV
29 IgM and IgG assays.

30 **Study design.** We assessed the reproducibility and cross-reactivity of both VIDAS assays and
31 the analytical sensitivity and linearity of the VIDAS IgG assay. We also tested the VIDAS and
32 comparator assays Wantai IgG and IgM on immunocompetent and immunocompromised
33 patients. Data were analysed according to the infectious profile, with samples from viremic
34 phase (HEV RNA/IgM positive) and post-viremic phase (HEV RNA negative, IgM positive)
35 infections, and uninfected patients (HEV RNA/IgM negative).

36 **Results.** Within-run reproducibility was <10% and between-run reproducibility was <12% for
37 both assays. We found no cross-reactivity, except for the VIDAS IgG assay in some patients
38 with HBV (1/10) or malaria (3/23) infections and for the VIDAS IgM assay in some HIV-
39 infected patients (1/10). The VIDAS IgG assay was linear over 0.10-10.0 U/mL. Analytical
40 sensitivity of the IgG assay was 0.71 IU/ml (probit analysis). The clinical sensitivity of the
41 VIDAS IgM assay was 97.65% for viremic samples (83/85) and 59.15% (42/71) for post-
42 viremic samples from immunocompetent patients. It was 78.95% (45/57) for acute phase
43 samples and 77.78% (28/36) for post-viremic samples from immunocompromised patients.
44 Specificity was excellent (>99%) in both populations.

45 **Conclusion.** The analytical and clinical performance of the new VIDAS® ANTI-HEV assays was
46 excellent. These rapid, automated assays for detecting HEV antibodies will strengthen the
47 arsenal for diagnosing HEV infections.

48 **1. Background.**

49 Hepatitis E virus (HEV) is a leading cause of viral hepatitis worldwide (1). Five major
50 genotypes within the *Orthohepevirus A* species, *Hepeviridae* family, are known to infect
51 humans (genotypes 1–4 and 7) (2). Genotypes 1 and 2 are restricted to humans and are
52 predominant in developing countries, where the virus is transmitted through faecally
53 contaminated drinking water. HEV genotypes 3 and 4 are predominant in industrialised
54 countries where zoonotic transmission occurs (2).

55 Hepatitis E virus typically causes an acute, self-limiting illness similar to other viral
56 hepatitis (3). Chronic cases of hepatitis E caused by infections with HEV genotypes 3, 4 and 7
57 have been reported in immunocompromised individuals, such as organ transplant recipients
58 (4-6), patients with haematological malignancies (7, 8) and individuals infected with HIV (9,
59 10).

60 HEV infections are diagnosed by laboratory testing since their clinical presentation
61 does not differ from that of other pathogens causing hepatitis (11). A combination of
62 serology (IgM and IgG tests) and nucleic acid assays is used (12). The brief window in which
63 HEV RNA can be detected in the serum or faeces of immunocompetent individuals is
64 confined to the acute phase of the disease. Several commercial serological HEV diagnostic
65 tests are currently available, but their sensitivities and specificities vary widely (13-18). One
66 recent evaluation of anti-HEV assays found that their IgM assay results agreed for only 71%
67 of suspected hepatitis E patients while the IgG assays agreed for only 70%. Their sensitivity
68 also varied (42 - 96%), which was reflected in the detection limits: up to 19-fold variations
69 for IgM and 17-fold for IgG (13). And none was automated.

70

71 **2. Objectives**

72 To evaluate the analytical and clinical performances of the new VIDAS® ANTI-HEV IgM
73 and IgG assays by testing samples from immunocompetent and immunocompromised
74 patients.

75

76 **3. Study design**

77 **3.1 Quality control materials and clinical samples**

78 The reproducibility of the two VIDAS® ANTI-HEV assays was determined using serum
79 samples according to the Clinical and Laboratory Standards Institute guidelines (CLSI) EP5-A3
80 guideline. Samples (3-4) covering the assay range were tested using 2 reagent lots in
81 duplicate, twice a day for 10 days, on 3 different VIDAS® instruments.

82 We assessed the cross-reactivity of the VIDAS® ANTI-HEV assays by testing samples
83 positive for IgM or IgG HAV, HBV, HCV, CMV, EBV, HIV, dengue or malaria infection. All these
84 samples tested negative for HEV IgG and IgM with the Wantai assays (Biologic Pharmacy
85 Enterprise, China).

86 The linearity of the VIDAS® ANTI-HEV IgG assay was evaluated according CLSI EP6-A
87 guidelines, using two pools of serum, one with a high and one with a low concentration of
88 HEV IgG (near the limits of the VIDAS® assay range). High and low samples were sequentially
89 mixed to generate 8 samples of intermediate concentrations. Each sample was tested in
90 triplicate with 2 reagent lots.

91 The analytical sensitivity of both the VIDAS and Wantai HEV IgG assays was assessed
92 by testing 20 replicates of each HEV IgG concentration (0.15 - 0.7 U/mL, 0.05 U/mL intervals)

93 prepared by diluting the WHO anti-HEV standard (NIBSC 95/584) in PBS containing 7.5%
94 bovine serum albumin.

95 The VIDAS® ANTI-HEV IgM and ANTI-HEV IgG clinical trial was a prospective
96 monocentric study. We tested 659 samples from immunocompetent and
97 immunocompromised patients (mean age: 49 years; range 1-91 years; male/female ratio:
98 1.01) characterized for hepatitis E (HEV RNA detection and Wantai HEV IgM assay). Data
99 were analysed with reference to the infectious profile, with samples from the viremic phase
100 (WantaiHEV IgM and HEV RNA positive; n= 142), post-viremic phase (Wantai IgM positive,
101 HEV RNA negative; n=107) and uninfected (HEV RNA negative and Wantai HEV IgM negative;
102 n=410). The samples were collected between September 2016 and June 2017 during
103 hospitalization and routine out-patient visits. Serum or EDTA–plasma samples were collected
104 prospectively: 86.8% were stored at –20°C and 13.2% were tested fresh.

105 We also tested patients infected with HEV genotype 1 (n=2), genotype 3-rabbit (n=2)
106 and genotype 4 (n=2).

107

108 **3.2 Laboratory Investigations**

109 The VIDAS® ANTI-HEV IgM and IgG assays (BioMérieux, France) used 100 µl of
110 samples (blood plasma or serum). Both these sandwich enzyme immunoassays were coated
111 with genotype 1 antigens. The VIDAS® ANTI-HEV IgM is a qualitative assay, the test value is
112 automatically calculated by the instrument as an index value. Results were considered to be
113 negative if their IgM index value was below the threshold value (set at 1.00).

114

115 The quantitative VIDAS® ANTI-HEV IgG measures the intensity of the fluorescence,
116 which is proportional to the concentration of HEV IgG in the sample. Test values are
117 automatically calculated by the instrument and expressed in U/mL. The VIDAS® ANTI-HEV
118 IgG assay has been standardized to internal reference calibrators titrated against the WHO
119 international standard NIBSC 95/584. Results were considered to be negative if the IgG was
120 below the threshold value (0.56 U/mL).

121 The Wantai HEV IgG and IgM EIA kits (Wantai Biologic Pharmacy Enterprise, Beijing,
122 China) were used according to the manufacturer's instructions. HEV RNA was tested using an
123 accredited ISO15189 RT-PCR assay (19).

124

125 **3.3 Statistical analysis.**

126 Variability is expressed as the standard deviation (SD) or the coefficient of variation (CV).
127 Linearity was evaluated as described in the CLSI protocol EP6-A with an accepted deviation
128 from linearity $\leq \pm 12\%$ over the entire measuring range. Probit analysis of data from serial
129 dilutions of the WHO standard was used to determine the analytical sensitivity of the HEV
130 IgG assays.

131

132 **4. Results**

133 **4.1 Analytical performances of the VIDAS® HEV assays**

134 *Reproducibility.* Within-run reproducibility was defined as the precision within-lot, within-
135 run and within-instrument. Between-run reproducibility included all variable factors: run,
136 day, and lot. The within-run reproducibility of the VIDAS® ANTI-HEV IgM assay yielded a CV
137 of 4.1 - 9.9%. Between-run reproducibility gave a CV of 7.4 - 13.0% (Table 1). The within-run
138 reproducibility of the VIDAS® IgG assay gave a CV of 4.5 - 7.8% and the between-run
139 reproducibility gave a CV of 9.1 - 11.9 % (Table 1).

140 *Cross-reactivity.* The cross-reactivity of the VIDAS® assays evaluated the potential
141 interference from other infections found in similar areas. All the samples tested negative for
142 HEV IgG and IgM with the Wantai assays. None cross-reacted in the VIDAS® IgM assay except
143 1/10 HIV-positive sample. Similarly, very few cross-reacted in the VIDAS® IgG assay: 1/10
144 HBV-positive sample and 3/23 malaria samples (Table 2).

145 *Linearity of the VIDAS® ANTI-HEV IgG.* Samples (n=8) were prepared by diluting the high-HEV
146 IgG sample with the low-concentration sample. A weighted linear regression between
147 observed and expected concentrations was linear from 0.10 to 10.0 U/mL with <10%
148 deviation from linearity (Figure 1). The slope of the regression line was 1.0199 ($R^2 = 0.99$).

149 *Analytical sensitivity of the HEV IgG assays.* Probit analyses of the replicates of the diluted
150 WHO standard gave an analytical sensitivity of 0.71 U/ml [95% CI: 0.69- 0.75] for the VIDAS®
151 IgG assay and 0.27 U/ml [95% CI : 0.24- 0.33] for the Wantai IgG assay.

152

153 **4.2 Clinical Performances of the VIDAS® ANTI-HEV IgM assay.**

154 *Sensitivity and specificity of the VIDAS® ANTI-HEV IgM in immunocompetent patients.* Of the
155 85 acute phase samples (viremic samples), 83 tested positive with the VIDAS® IgM
156 (sensitivity: 97.65%; 95%CI: 91.76 - 99.71%). The 71 Wantai IgM-positive HEV RNA-negative
157 samples (post-viremic) included 42 positive and 29 negative (sensitivity: 59.15%; 95%CI:
158 47.54 - 69.83%). These 29 samples that tested negative with the VIDAS® IgM assay included
159 25 that were positive with the VIDAS ANTI-HEV IgG assay. Only 2 of the 303 negative
160 samples tested positive with the VIDAS IgM (specificity: 99.34%; 95%CI: 97.64 - 99.92%).

161 *Sensitivity and specificity of the VIDAS® ANTI-HEV IgM in immunocompromised patients.* The
162 57 acute phase samples included 45 that tested positive with the VIDAS® IgM assay
163 (sensitivity: 78.95%; 95%CI: 66.71 - 87.53%). The 36 Wantai IgM-positive/HEV RNA-negative
164 samples included 28 that tested positive and 8 that tested negative (sensitivity: 77.78;
165 95%CI: 61.92 - 88.28%). These 8 VIDAS® IgM assay-negative samples included 5 that were
166 VIDAS IgG-positive. The 107 negative samples included one that was VIDAS IgM-positive
167 (specificity: 99.07%; 95%CI: 94.90 - 99.98%).

168

169 **4.3 Clinical Performance of VIDAS® ANTI-HEV IgG assay.**

170 *Sensitivity and specificity of the VIDAS® ANTI-HEV IgG in immunocompetent patients.* The 85
171 viremic samples included 81 that tested positive with the VIDAS IgG (sensitivity: 95.29%; -
172 95%CI: 88.39 - 98.70%). Three of the 4 negative VIDAS IgG samples were also negative with
173 the Wantai IgG assay. Similarly, 65 of the 71 post-viremic samples tested positive and 6
174 tested negative (sensitivity: 91.55%; 95%CI: 82.76 - 96.07%). Three of these 6 negative
175 samples also tested negative with the Wantai IgG assay. The 303 negative samples included
176 10 that tested positive with the VIDAS IgG (specificity: 96.70%; 95%CI: 94.01 - 98.41%).

177 Three of these 10 samples that were positive with the VIDAS® IgG assay were also positive
178 with the Wantai IgG assay.

179 *Sensitivity and specificity of the VIDAS® ANTI-HEV IgG in immunocompromised patients.* The
180 57 viremic phase samples included 38 that tested positive with the VIDAS ANTI-HEV IgG
181 (sensitivity: 66.67%; 95%CI: 53.72 – 77.51%). The 36 post-viremic samples included 32 that
182 tested positive and 4 that tested negative (sensitivity: 88.89%; 95%CI: 74.69– 95.59%). The
183 107 negative samples included 4 that tested positive with the VIDAS IgG (specificity: 96.26%;
184 95%CI: 90.70 – 98.97%).

185 **4.4 HEV antibodies detection in HEV genotype 1, 3-rabbit and 4 infections**

186 All the samples from patients with HEV genotype 1, 3-rabbit and 4 infections were positive
187 for HEV IgG and IgM using both the Wantai and VIDAS® assays.

188

189 **5. Discussion**

190 Our evaluation of HEV IgM and HEV IgG assays developed for the VIDAS® system in
191 immunocompetent and immunocompromised patients indicated that they were very
192 sensitive for samples collected at the viremic phase of infection and extremely specific.

193 Several serological anti-HEV diagnostic tests have been evaluated recently. Their
194 sensitivities seem to vary widely, which has complicated data interpretation (13, 15, 17, 20,
195 21). The present clinical evaluation of the VIDAS® IgM assay indicates that it is very sensitive
196 (97.65%) in acutely infected (HEV RNA positive) immunocompetent patients and less
197 (78.95%) in immunocompromised patients. These results agree well with those of HEV IgM

198 assays evaluated in industrialized countries where genotype 3 is prevalent (14, 15, 22, 23).
199 The VIDAS IgM assay coated with genotype 1 antigens was equally sensitive for detecting
200 antibodies in genotype 3-infected individuals, consistent with other studies (14, 15, 24, 25).
201 However, the VIDAS IgM assay was less sensitive (only 59.15%) when tested on Wantai HEV
202 IgM-positive/HEV RNA-negative samples from immunocompetent patients. Thus, the VIDAS
203 IgM assay is less effective than the Wantai assay for detecting low concentrations of HEV
204 IgM. As the concentration of HEV IgM decreases with time and becomes undetectable 4 to 6
205 months after an acute infection (26), it may become undetectable earlier after an acute
206 infection with the VIDAS assay than with the Wantai. Norder et al. found that the DiaPro
207 assay remained positive for the longest after the onset of an HEV infection (13). Therefore,
208 the cut-off value determined by the manufacturer may also influence the assay sensitivity
209 (15). This restricted ability to detect long-lasting HEV IgM may help in patient management
210 as the clinician can rely on the HEV IgM assay for diagnosing an acute hepatitis E infection.
211 The specificity of the VIDAS IgM assay was also excellent in agreement with the reported
212 specificities of IgM assays (13, 14, 22, 23). Its cross-reactivity with interfering infections was
213 also very limited. Anti-HEV IgM assays may suffer from a high proportion of false-positive
214 reactions due to EBV or CMV infections (27-29), but this was not a feature of the VIDAS®
215 ANTI-HEV IgM assay in our hands.

216

217 The analytical sensitivities HEV IgG assays also vary, which strongly influences the
218 seroprevalence rates (16, 21, 30, 31). Evaluations of the analytical sensitivity of HEV IgG
219 assays have found differences: from 0.2 U/ml (Wantai assay) to 6.31 U/ml (Mikrogen assay,
220 old version) (13-15). The Wantai IgG analytical sensitivity varies from 0.2 to 0.4 U/ml (13-15),
221 which is similar to the results found for the Wantai assay in this study. The VIDAS® ANTI-HEV

222 IgG assay appears to perform as well as other HEV IgG assays (analytical sensitivity: 0.71
223 U/ml). The clinical sensitivity of the VIDAS® IgG assay was 95.29% in immunocompetent
224 patients and 66.67% in immunocompromised patients, in good agreement with previously
225 reported data for the Wantai IgG assay in HEV RNA positiveve patients: sensitivity 93.2% in
226 immunocompetent and 45% in immunocompromised (22). The VIDAS IgG assay is also a
227 quantitative assay that is linear up to 10 U/ml. Only the Euroimmun HEV IgG assay gives
228 quantitative results based on the WHO standard validated by the manufacturer. According
229 to the package insert, the Euroimmun HEV IgG assay is linear up to 25 U/ml, but it has a
230 higher limit of detection (1.5 U/ml) (15).

231 The VIDAS IgG assay was also highly specific, with values similar to those reported for
232 other HEV IgG assays (13, 14, 20, 22). It also displayed very limited cross-reactivity with
233 interfering infections, as did VIDAS IgG prototypes (32). It cross-reacted in samples from 3
234 patients with malaria. However, this diagnosis is rarely performed for patients who have not
235 traveled abroad in European countries.

236 We also assessed the ability of the VIDAS® assays to detect HEV antibodies in patients
237 infected with genotype 1, genotype 3-rabbit or genotype 4. The few samples tested
238 indicated that both VIDAS® assays to detected HEV antibodies with the same spectrum as
239 the Wantai assay. Although the anti-HEV assays use genotype 1 antigens, they detected
240 antibodies directed against other genotypes because of the considerable cross-reactivity
241 between the genotypes, which is consistent with HEV having a single serotype (33).

242 The ANTI-HEV VIDAS® assays can be performed on automated VIDAS® instruments
243 like the mini-VIDAS® or the VIDAS 3® automat. This latter immunoanalyzer is a fully
244 automated benchtop immunoassay system with full traceability. Its reduced hands-on time
245 from the sample to the result (<40min) enables efficient management of emergency tests.

246 Several immunochromatographic rapid tests are available for rapidly detecting HEV IgM (15,
247 23) but they are relatively insensitive (90% in immunocompetent patients) and interpreting
248 the results is sometimes difficult due to very faint bands and great dependence on the
249 operator.

250 Thus, the VIDAS® system provides sensitive, specific automated tests that are
251 suitable for the rapid diagnosis of acute hepatitis E and are useful addition to the arsenal for
252 diagnosing HEV infections. The VIDAS® ANTI-HEV IgG assay is a quantitative assay with a low
253 detection limit that is appropriate for determining the HEV status in epidemiological studies.

254

255 **Competing interests:** FA, RJ, AA, SB, SL, and JI: none. NG, EE, SB, LM, ALT, DP, SD, FL, CP, CT
256 and JMD, are employed by Biomérieux.

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259 **Ethical statement.** Patient records and information were anonymized and de-identified prior
260 to analysis. Ethical approval was obtained from the French “Comité de Protection des
261 Personnes Nord Ouest I” (N°2017-A00196-47).

262

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349 **Figure legends**

350 Figure 1. Linearity of the VIDAS® ANTI-HEV IgG assay.

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Figure 1

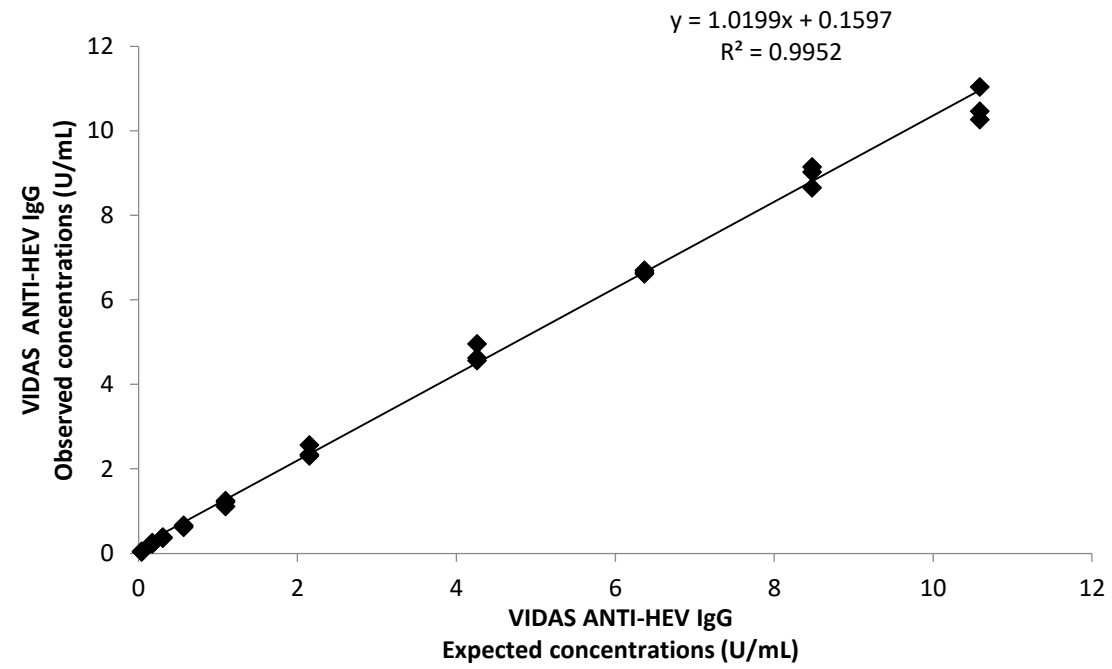


Table 1: Variability of the VIDAS® anti-HEV IgM and IgG assays

| Test | Sample | Mean value | Repeatability | Reproducibility | Reproducibility |
|------------|----------|------------|---------------|-------------------|-------------------|
| | | | within-run | Within-lot | Between-lot |
| | | | CV (%) | Within-instrument | Within-instrument |
| | | | | CV (%) | CV (%) |
| IgM | Sample 1 | 0.91 | 9.9 | 12.3 | 13.0 |
| | Sample 2 | 1.60 | 4.1 | 7.0 | 7.4 |
| | Sample 3 | 13.15 | 5.1 | 7.4 | 7.4 |
| IgG | Sample 1 | 0.14 | 7.8 | 11.9 | 11.9 |
| | Sample 2 | 0.64 | 5.1 | 8.6 | 9.1 |
| | Sample 3 | 2.88 | 4.5 | 7.5 | 10.8 |
| | Sample 4 | 6.97 | 4.6 | 7.8 | 9.7 |

Table 2: Cross reactivity study for the VIDAS® anti-HEV assays

| Organism/Condition | VIDAS® ANTI-HEV IgM | | | | VIDAS® ANTI-HEV IgG | | | |
|-----------------------------------|---------------------|----------|----------|--------------------|---------------------|----------|----------|--------------------|
| | N | Positive | Negative | % Cross Reactivity | N | Positive | Negative | % Cross Reactivity |
| Anti-Hepatitis A virus | 10 | 0 | 10 | 0% | 10 | 0 | 10 | 0% |
| Anti-Hepatitis B virus | 14 | 0 | 14 | 0% | 10 | 1 | 9 | 10% |
| Anti-Hepatitis C virus | 12 | 0 | 12 | 0% | 10 | 0 | 10 | 0% |
| Anti-Cytomegalovirus | 13 | 0 | 13 | 0% | 10 | 0 | 10 | 0% |
| Anti-Epstein Barr Virus | 10 | 0 | 10 | 0% | 10 | 0 | 10 | 0% |
| Anti-Dengue virus | 12 | 0 | 12 | 0% | 10 | 0 | 10 | 0% |
| Anti-Human Immunodeficiency virus | 10 | 1 | 10 | 10% | 12 | 0 | 12 | 0% |
| Malaria | 10 | 0 | 10 | 0% | 26 | 3 | 23 | 11% |